

# Post-harvest storage effects on guayule latex quality from agronomic trials

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## Abstract

Current guayule commercialization efforts are based upon the production of hypoallergenic latex. The objective of this study was to determine how latex yield and quality are affected by post-harvest plant storage in order to provide flexibility in the harvesting, chipping, and processing steps for guayule latex. The experiments were conducted on two lines (11591 and AZ-2) at the University of Arizona Maricopa Agricultural Center, from March 2001, through December 2004. Weight-average molecular weight of guayule latex increased as the plants aged from 1.7 to 2.7 years. Guayule latex quality was affected by shrub storage conditions, and addition of moisture extended the storage time for guayule shrub without negatively impacting molecular weight for both varieties. However, under extreme conditions, for example, high temperatures and extended dry storage times, polymer molecular weight reduction of up to 30% occurred.

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## 1. Introduction

Guayule, *Parthenium argentatum* gray, is a woody desert shrub native to northern Mexico and the Big Bend, Texas region of the United States. Guayule synthesizes high molecular weight *cis*-1,4-poly(isoprene) known as natural rubber. It has been the subject of considerable research, particularly during times of short supply or price pressure for natural rubber produced by *Hevea brasiliensis* (Whitworth and Whitehead, 1991; Cornish

and Schloman, 2004). Guayule latex contains no soluble protein, is low in hydrophobic rubber particle-bound proteins, and does not cross-react with Type I latex allergy (Siler and Cornish, 1994; Siler and Cornish, 1995; Siler et al., 1996). Thus, guayule latex provides a source of hypoallergenic latex suitable for the manufacture of medical products, and is being commercially developed on this basis.

Commercial reintroduction of guayule rubber requires agronomic and industrial processing practices that minimize the loss of quality and quantity of latex between the time the shrub is harvested until the latex is delivered to the product manufacturer. Once harvested, natural processes occurring within the shrub that protect the rubber from oxidative degradation are progressively diminished over time. It is not always practical to

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process shrub immediately after harvest, and in some cases it may not be desirable. In fact, prior to 1952, field curing of guayule shrub, from 10 to 45 days, was an accepted agronomic practice (Taylor and Chubb, 1952), considered essential to reduce the weight of material to be transported, and to recover the maximum amount of rubber in the milling (solid rubber extraction) process.

According to previous workers field storage most likely influences the rubber content, especially in latex extractable form, and the polymer molecular weight, due to degradative processes naturally occurring after plant harvest. Various effects of post-harvest storage of guayule shrubs, chipped material, and homogenate have been studied by workers using a range of laboratory and field conditions (Black et al., 1986; Schloman et al., 1986; Estilai and Hammerstrand, 1989; Dierig et al., 1990; Nakayama and Coates, 1996; Cornish et al., 2000; Coffelt et al., 2005). These workers clearly showed that long term, high temperature field storage adversely affects rubber content, especially latex content, and rubber quality, as measured by polymer molecular weight. Degradation was considered to be due to a combination of oxidative and enzymatic processes. Post-harvest degradation was genotype dependent and, as such, may respond to improvements via plant breeding (Estilai and Hammerstrand, 1989; Dierig et al., 1990).

Economical recovery of rubber from harvested guayule requires a thorough understanding of the influence of storage variables on recovery of natural rubber in its liquid latex form. Seasonal effects, variety effects, and moisture levels, as well as various agronomic practices, must be well understood to maximize productivity. The objective of our present study was to determine how latex yield and quality are affected by post-harvest plant storage in order to provide flexibility in the harvesting, chipping, and downstream processing steps for guayule latex.

## 2. Materials and methods

### 2.1. Plant establishment

The experiment was conducted to determine the effect of shrub storage method following harvest and prior to chipping on the latex yield and quality of two lines (11591 and AZ-2). The plants used in this study were transplanted at the University of Arizona Maricopa Agricultural Center, March 2001. Harvest took place three times per year (March, July, November) for two years beginning in November 2002 when the plants were 1.7 years old.

### 2.2. Storage treatments

Each line was subjected to ten storage treatments which consisted of various combinations of drying and wetting periods (Table 1). Drying periods consisted of placing the harvested shrub in the shade, under ambient temperature conditions. Moist storage was achieved by light water misting of the shrubs, typically 2–3 min per day, under the same shaded, ambient temperatures. The experiment was a randomized complete block design with four replicates. Three years of storage experiments (2002, 2003, 2004) were included in this study for a total of 320 samples. Additional details for the experimental design have been reported by Coffelt et al. (2005).

### 2.3. Harvest and processing

Plants were harvested using the method developed by Coffelt and Nakayama (2004) and were processed immediately (control, treatment 1) or stored prior to chipping as described in Table 1. A six-step protocol was used to process the shrubs: (1) plants were cut in the field close to ground level (–50 mm); (2) plants were transported from the field to the chipping area as soon as possible in bags; (3) fresh weight was obtained for each sample; (4) the sample was processed through the chipper and fresh weight of the chipped material recorded; (5) ammoniated antioxidant solution (0.2% sodium sulfite in distilled water at a pH of about 11) was added so that fresh weight of plant material collected and antioxidant solution were in a 1:1 ratio; (6) the antioxidant solution was thoroughly mixed with the plant material. Steps 2–6 were done in less than 3 h to minimize latex loss. The freshly chipped mixture was stored at 4–10 °C prior to laboratory analysis for latex.

Table 1  
Shrub storage conditions

| Treatment | Storage conditions                                 |
|-----------|--|
| 1         | Plants chipped immediately after harvest           |
| 2         | 7 days dry   |
| 3         | 7 days moist                                       |
| 4         | 14 days moist                                      |
| 5         | 7 days dry, then 7 days moist                      |
| 6         | 21 days moist                                      |
| 7         | 14 days dry, then 7 days moist                     |
| 8         | 28 days moist                                      |
| 9         | 21 days dry, then 7 days moist                     |
| 10        | 7 days dry, 7 days moist, 7 days dry, 7 days moist |

*Note:* All plants stored in the shade at ambient temperature, seasonally variable.

## 2.4. Latex recovery

Subsamples (0.5–1.0 kg) of antioxidant treated fresh plant chip mixture were placed in plastic bags, packed in ice, and shipped overnight in coolers to the USDA-Western Regional Research Center in Albany, California, for further processing and analysis. At the laboratory, each sample was weighed, then placed into a one gallon Waring blender and covered with a measured amount of antioxidant solution (AAO: 0.1%  $\text{Na}_2\text{SO}_3$ , pH 9). Samples were ground for 60 s; the filtrate pressed through four layers of cheesecloth and collected. A hand-powdered hydraulic press with a 1 mm stainless steel mesh screen was used to press the mixture such that the residual bagasse was moderately damp. The filtrate was adjusted to pH 10 with ammonium hydroxide, and the volume of homogenate recorded. Homogenate was centrifuged in a Sorvall RC-5B or RC 5-C bucket rotor for 40 min at 7000 rpm to sediment non-latex solids, and the supernatant (clarified homogenate) was decanted. Centrifugation was repeated twice. Three volumes of 0.1% ammonium alginate (in AAO) was added to one volume of clarified homogenate or pooled light phase, in a separation vessel and the concentration adjusted to 0.1% alginate. The contents of the vessel were swirled gently to mix the solution without forming bubbles and stored overnight. A layer of latex formed on top of the alginate solution. The supernatant solution was drained from the bottom of the separation vessel and the latex layer resuspended in 0.1% alginate (1:5, v/v) and the suspension gently swirled. The latex layer was then repeatedly washed with 0.05% alginate until reaching the point when no discoloration of the supernatant was apparent (3–6 washes). The purified latex was then transferred to a suitable tube or bottle, overlaid with nitrogen gas and stored at 4 °C until analyses were performed. The latex storage conditions used resulted in varying levels of latex coagulation: mild to moderate, and, in some cases, severe coagulation. In most cases sufficient liquid latex remained for particle size and protein assay measurements. For molecular weight determinations, rubber fractions from both fresh latex and coagulate were fully soluble in the organic solvent used.

## 2.5. Particle size

Particle size of the latex was determined using a Horiba LA-900 Laser Light Diffraction Particle Size Distribution Analyzer according to the manufacturer's instructions (Horiba, 1996).

## 2.6. Protein determination

The amount of protein in the latex was determined using a Pierce micro-BCA assay kit. This procedure involved the solubilization of protein from latex with sodium dodecyl sulfate. The aqueous protein was removed from the latex, precipitated with trichloroacetic acid, and re-suspended in sodium hydroxide solution. The solubilized protein was then mixed with bicinchoninic acid reagent and the protein level quantified against a series of bovine serum albumin standards using an Ultraspec-3000 spectrophotometer. Details on the procedure can be found in Siler and Cornish (1995).

## 2.7. Rubber molecular weight

Molecular characteristics of rubber in solution were determined by size exclusion chromatography with multi-angle laser light scattering (SEC-MALLS). Fifteen microliter liquid latex was dissolved overnight in 2–5 ml of 0.2  $\mu\text{m}$  filtered tetrahydrofuran (THF) in 8 ml borosilicate vials with Teflon coated lids. The next day, the sample solution was filtered through 1.6  $\mu\text{m}$  glass microfiber/polypropylene housing (GF/A) Whatman syringe filter into 12 mm  $\times$  32 mm clear borosilicate screw-cap vials w/poly(tetrafluoroethylene) (PTFE) septa. Molecular weights and their distributions were determined using a Hewlett-Packard 1100 series high pressure liquid chromatograph (HPLC), coupled to multi-angle laser light scattering (DAWN DSP Laser Photometer, Wyatt Technologies, Santa Barbara, CA) and refractive index (HP1047) detectors. For each sample, following a THF blank run, a 50  $\mu\text{l}$  subsample was injected and run through a Phenogel 5 m Linear/Mixed Guard Column (Phenomenex) and two Polymer Labs gel 10  $\mu\text{m}$  mixed-B exclusion columns connected in series. The flow rate was 1 ml/min, and the column temperature was 35 °C.

## 3. Results

### 3.1. Particle size

Natural rubber latex is a stable aqueous dispersion of small rubber particles, each surrounded by a monolayer bio-membrane containing a species-specific complement of proteins and lipids (Siler et al., 1997; Cornish et al., 1999). The size of latex particles is always represented by a distribution, varies by species, and at times within a species (Cornish, 2001). The guayule latex

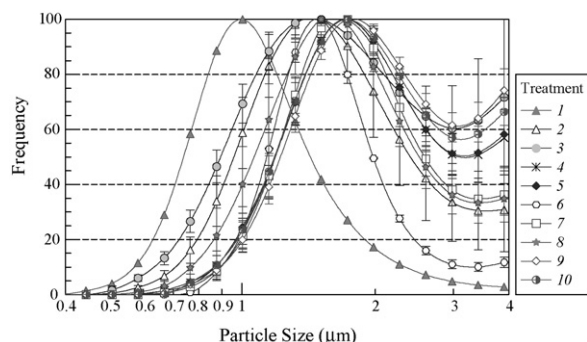


Fig. 1. Particle size distribution for guayule latex recovered from the November 2002 harvest of variety AZ-2 (plant age 1.7 years). Control is Treatment 1.

particle size distributions for control and experimental treatments for the first (November 2002) harvest of AZ-2 are shown in Fig. 1. All data were normalized to 100 relative frequency for ease of comparison, a natural spline interpolation was used to smooth the curves, and errors bars represent the standard error of replicate measurements where available. The first harvest of 1.7-year-old plants shown in Fig. 1 is typical of the results found in 5 of 6 cases (2 lines  $\times$  3 harvests). The control sample (Treatment 1) shows an average particle size of about 1  $\mu\text{m}$ , with a distribution ranging from 0.4 to  $>4 \mu\text{m}$ , skewed toward larger particles. Most of the rest of the samples are clustered around a mean particle size of just under 2  $\mu\text{m}$ , with a few samples in between. The increase in frequency at higher diameters ( $>3 \mu\text{m}$ ) of many of the samples is attributed to the presence of coagulate. In fact, many of the stored latex samples showed significant levels of coagulation; despite filtering prior to measurements, peaks representing coagulated latex appeared frequently. Control samples were usually more resistant to the tendency to coagulate. In general, storage condition severity increases with Treatment #2 to #10. No trends were observed correlating individual storage treatments with particle size or size distribution for the experimental treatments. Similar results were found for the remaining harvests: low average particle size for the control, and a cluster of higher particle sizes for all experimental treatments. The average particle size values were similar for all lattices tested, regardless of line, plant age, or harvest season. Table 2 includes combined data indicating low variation of control average particle size around 1.0  $\mu\text{m}$ , and all latex from stored shrubs over 1.5  $\mu\text{m}$ . The November 2003 harvest of 11591 is the single exception to these observations; all treatments including the control are relatively tightly clustered around a mean particle size of about 1.5  $\mu\text{m}$ .

Table 2

Combined average particle size of latex

| Storage treatment | Average ( $\mu\text{m}$ ) | Standard error |
|-------------------|---------------------------|----------------|
| 1                 | 1.07                      | 0.14           |
| 2                 | 1.59                      | 0.07           |
| 3                 | 1.66                      | 0.08           |
| 4                 | 1.69                      | 0.04           |
| 5                 | 1.73                      | 0.01           |
| 6                 | 1.62                      | 0.05           |
| 7                 | 1.59                      | 0.07           |
| 8                 | 1.69                      | 0.08           |
| 9                 | 1.45                      | 0.08           |
| 10                | 1.53                      | 0.10           |

Averages represent both lines (AZ-2 and 11591) for three harvests, November 2002, November 2003, and March 2004.

### 3.2. Protein assay

Results for total protein, expressed as micrograms protein per milligram dry weight rubber, appear in Table 2. The data represent the first (November 2002) and second last (March 2004) harvests. No differences were found for protein levels for different storage treatments for any harvest. The Table 2 data was, therefore, combined per harvest and sorted by line. Protein levels for AZ-2 are higher than 11591 for the November 2002 harvest, and dropped to lower values by the March 2004 harvest. Those differences are significant only within  $\pm 1$  standard error. The average total protein content for 11591 is consistent between harvests. Combined data for both harvests indicate total protein in these guayule rubber samples is about 5.7  $\mu\text{g}/\text{mg}$  dry rubber, with no significant difference between lines Table 3.

### 3.3. Molecular weight

The molecular weight of any polymer is expressed as an average of a distribution, and different averages weight low or high fractions more heavily. Size Exclusion Chromatography provides information about the size and shape of polymer molecules and has proven quite useful in characterizing *cis*-1,4-polyisoprene produced by biosynthesis (Cornish and Schloman, 2004). Concerns about the effect of shrub storage on latex quality are related to polymer degradation, i.e., chain length reduction. The weight average molecular weight,  $M_w$ , is most sensitive to the longer, i.e. higher molecular weight chains in the  $M_w$  distribution. Therefore, one might expect the weight-average molecular weight to be more sensitive to degradation effects, as the longest chains undergo cleavage. In our analysis weight average, number average, and polydispersity measures were

Table 3  
Combined protein content at harvest for lines AZ-2 and 11595

| Harvest date  | Variety and total protein ( $\mu\text{g}/\text{mg}$ dry weight rubber) |                |          |         |                |          |
|---------------|--|----------------|----------|---------|----------------|----------|
|               | AZ-2   |                |          | 11591   |                |          |
|               | Average  | Standard error | <i>N</i> | Average | Standard error | <i>N</i> |
| November 2002 | 8.45   | 1.51           | 16       | 5.93    | 1.62           | 13       |
| March 2004    | 3.39   | 0.79           | 18       | 5.36    | 1.09           | 24       |
| All Data      | 5.85   | 0.90           | 35       | 5.57    | 0.87           | 38       |

investigated, and, indeed, the  $M_w$  values showed the greatest sensitivity to experimental conditions, as found by previous workers (Black et al., 1986; Schloman et al., 1986; Estilai and Hammerstrand, 1989; Dierig et al., 1990). Therefore, the data presented will focus on changes to weight-average molecular weight throughout this section.

### 3.3.1. Plant age

The average  $M_w$  values for the first (November 2002, 1.7-year-old plants) and the last (3.3-year-old plants) harvests are shown in Fig. 2. For nearly every case (92%) where comparable data exist, the  $M_w$  values are higher in July 2004 than November 2002. Additional data (not shown) indicate that the average  $M_w$  increased until the plants were 2.7 years old. By the fifth harvest (3.0 years old) the average  $M_w$  showed little change.

### 3.3.2. Moist versus dry storage/summer versus winter harvest

The effect of seven days dry or moist storage can be made by a comparison of Treatment #1 (control) with #2 (7 days dry) and #3 (7 days moist). Fig. 3a–b illustrate that comparison graphically. No significant differences were detected for any of the comparisons (Data sets are incomplete where insufficient latex could be recovered, or where the amount of latex recovered precluded multiple tests). The summer harvest, with highest temperature conditions, provides the best environment for thermal degradation. But molecular weights of rubber recovered after seven days summer storage were within statistical equivalence of controls. Dry storage treatments beyond seven days led to significant latex losses (see Coffelt et al. (2005), also Fig. 5 and discussion). For the two summer harvests (and one March harvest) it was not possible to recover sufficient latex for testing from shrub stored beyond seven days.

A systematic study of the effect of time under moist storage conditions is illustrated by a comparison of Treat-

ments # 1 (control), #3 (7 days), #4 (14 days), #6 (21 days), and #8 (28 days). Fig. 4 plots  $M_w$  as a function of days of moist storage for two harvests. Overall, moist storage appears to be quite effective at preserving the molecular weight of *cis*-1,4-polyisoprene rubber in guayule shrub. Significant reductions in  $M_w$  are not seen until 28 days post-harvest, for March 2003 and July 2003, both quite warm months in Arizona.

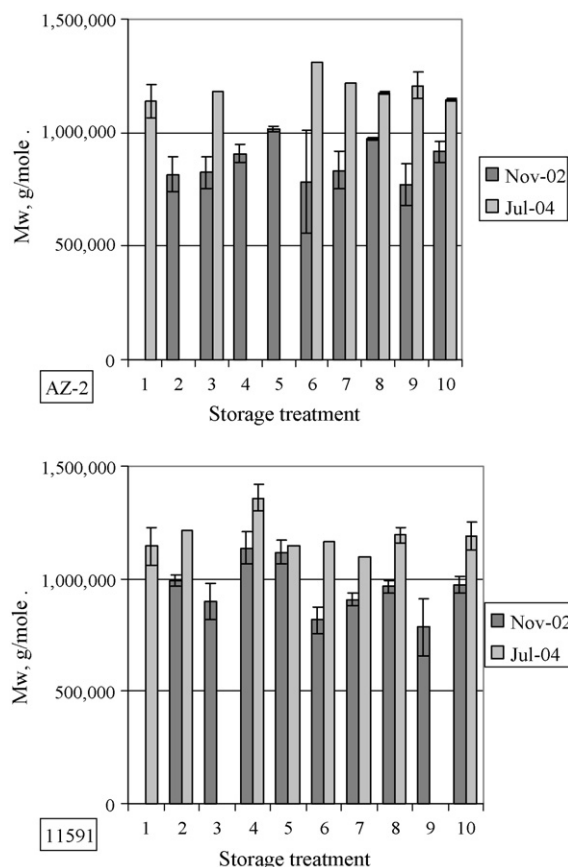


Fig. 2. Average molecular weight,  $M_w$ , for rubber recovered from guayule latex following shrub storage under various treatments. Comparison of first (November 2002, 1.7 years old plants) and last (July 2004, 3.3 years old plants) harvests.



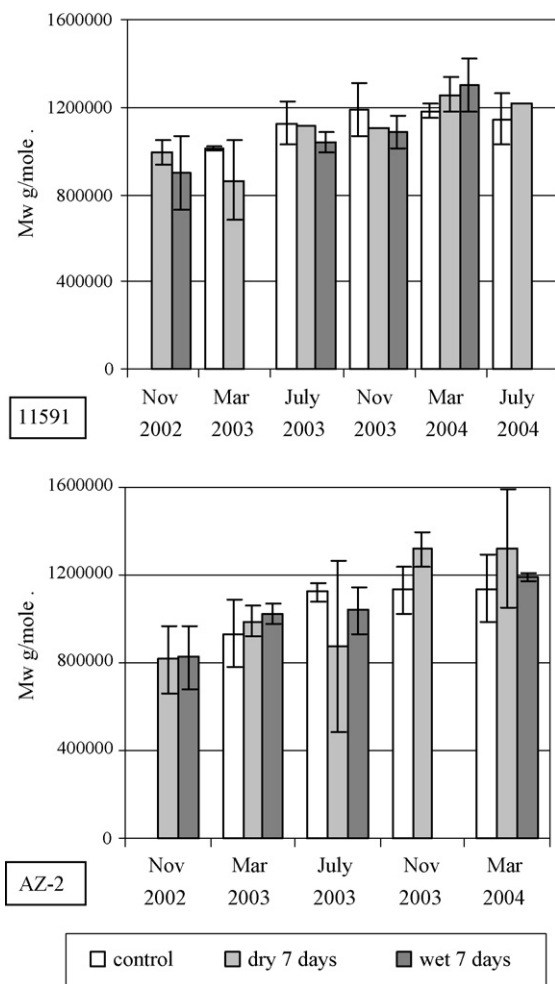


Fig. 3. Average molecular weight,  $M_w$ , for rubber recovered from guayule latex following 0–7 days shrub storage. Plant ages from 1.7 year (November 2002) to 3.3 year (March 2004).

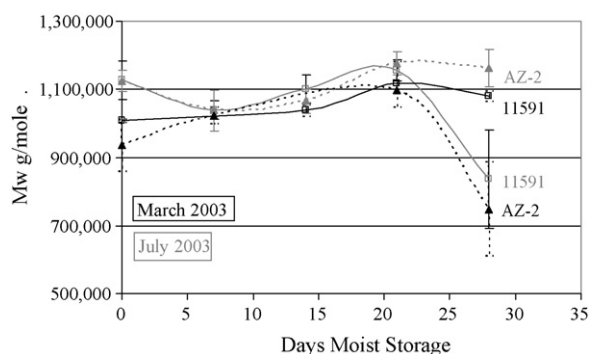


Fig. 4. Average molecular weight,  $M_w$ , for rubber recovered from guayule latex following 0–26 days shrub storage under moist conditions. Plant ages are 2.0 year (March 2003) to 2.3 year (July 2003).

#### 4. Discussion

Latex quality can be described by particle size, protein content, and rubber molecular weight, among other factors. These characteristics were measured over a three and a half year period for two lines of harvested guayule shrub subjected to various storage treatments. Latex total protein content showed no significant differences attributable to post-harvest storage conditions. Latex particle size mean values for control samples were lower than typically measured. Controls for this study averaged  $1.07 \mu\text{m}$ , and were as low as  $0.75 \mu\text{m}$ . Typical results for fresh guayule latex in our laboratory are closer to  $1.5 \mu\text{m}$ , and results from a recent pre-commercial sample (McMahan et al., 2005) averaged  $1.32 \mu\text{m}$ . One possible explanation for differences lies in the process used: high energy chipping of shrub could promote particle coagulation, especially for a dry chip process. The larger, older particles, residing in plant cell vacuoles, might be preferentially coagulated in this process, which would lead to a lower mean particle size.

For five of six harvests, shrub storage caused a shift to larger average particle size. The minimum shrub storage time in this study was 7 days. More severe treatments showed similar shift in mean particle size compared to less severe treatments, indicating that particle size changes were mostly complete after seven days. This implies dehydration, rather than degradation, is responsible for particle size changes, through coagulation effects. Dehydration of guayule branches is known to have a dramatic influence on the extractable latex (Cornish et al., 2000). In that study, losses were noted at 80% relative water content. Even hydrated branches stored at  $4^\circ\text{C}$  lost latex content due to a combination of solid rubber conversion (coagulation) and degradation. In the present study, only the freshest (control) shrubs, processed as quickly as possible, had the best protection from dehydration. The results presented here suggest that the latex recovered from stored shrubs has already begun to coagulate, that early particle coalescence still allows for recover as latex, and that the misting process used to help retain moisture does not prevent that coagulation.

Coffelt et al. (2005) has separately reported that moist storage preserved or increased latex concentration and latex yield over freshly harvested shrub for the plants used in this study. In contrast, dry storage were generally lower than freshly harvested shrub in latex concentration and yield. The differences in latex concentration measured were significant and substantial (Coffelt et al., 2005), and are contrasted with  $M_w$  effects in Fig. 5a–b for two harvests. Large differences in latex concentration, especially under dry storage conditions in March and

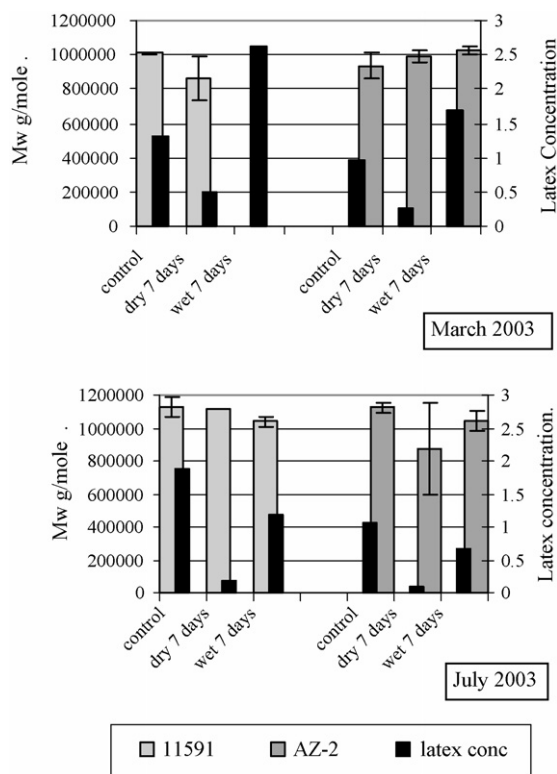


Fig. 5. Latex concentration and average molecular weight,  $M_w$ , for rubber recovered from guayule latex following 0–7 days shrub storage. Latex concentration data is taken from Coffelt et al. (2005).

July of 2003, are not reflected by accompanying changes in  $M_w$ . This confirms that the source of the latex loss is not be due to degradation. Because this study recovered polymer only in the latex form, it is likely that coagulation of latex contributed to concentration losses, and that the latex recovered, while lower in quantity, has suffered relatively little degradation.

For this study, once degradation took place it did so quickly. For example, moist storage up to 21 days (Fig. 4) showed high molecular weights for March and July harvests for both lines. Over the next seven days (day 21 to day 28),  $M_w$  was reduced by approximately 25%. For unprotected rubber, microbial, enzymatic, or oxidative degradation processes could each proceed quickly once threshold conditions are reached.

It has been suggested that the breakdown of the rubber molecule occurs as soon as the plant is harvested and that degradation may occur both oxidatively and enzymatically. When Taylor and Chubb (1952) compared fresh and stored shrub, from Variety 593 for rubber quality, the fresh shrub, which was processed with a minimum delay after harvest, yielded the maximum quantity of high molecular weight rubber. Guayule shrub which was

field cured for seven days, baled with the leaves, then processed, showed a ~30% molecular weight reduction (by solution viscometry). In our study seven days storage showed no evidence of degradation. The key difference between our work and that of Taylor and Chubb is recovery of the latex versus recovery of the rubber. Solvent-recovered rubber includes that fraction lost to latex through coagulation. Perhaps the lipid membrane surrounding latex particles provides protection from degradation for the latex fractions. The coagulated rubber, still stored by the plant, may be more susceptible to degradation. It is also probable that the advancement of shrub quality in the lines used in the present study have resulted in a plant that is indeed more resistant to polymer degradation, regardless of the mechanism. A study conducted in the early 1980s (Estilai and Hammerstrand, 1989) of field storage of 20 different guayule varieties found distinct genotype-dependant differences with respect to rubber content and molecular weight. The varieties used in our study present a view of plants with considerable resistance to polymer degradation. Molecular weight degradation in the Arizona summer was not significant, especially under moist conditions, for up to 21 days. This study confirms that proper storage of guayule plants post-harvest can maintain latex quality for up to four weeks.

Coffelt et al.'s (2005) data showed moist storage can preserve latex yield (concentration), and under mild (spring) ambient temperatures result in little or no latex loss at up to seven days storage. The same shrubs showed no significant loss in quality, i.e., polymer degradation, over that same time period. Further, when latex losses did occur (summer temperatures, dry storage) the quality of latex recovered was still good. These findings are significant in that they suggests shrub should be kept moist when stored, that it can be stored up to seven days in mild weather without loss in quantity or quality, and that if longer storage periods are needed, or higher temperatures encountered, some latex will be lost. The recoverable latex, while lower in quantity, should be nevertheless of good quality.

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